REMARKS

Status of the Claims

Claims 1-31 were presented in a request for continued examination. Claims 1-11, 18, and 20-31 were previously withdrawn in response to a restriction requirement. Claims 23-31 have now been canceled. Claims 12-17 and 19 were examined and were rejected. Claim 12 was amended. The amendment removes language that the Examiner has indicated does not limit the invention as claimed, and enhances clarity. The Amendment also removes a phrase excluding BCG from the claim scope: this exclusion was not in the original claim, so it adds no new matter. In view of the remarks below it should be clear why this language is not necessary to distinguish the claimed invention from the prior art, and has thus been removed. Claim 1 is withdrawn but was identified by the Examiner as a method claim related as method of use of the composition claims under examination; it is now amended to include all limitations of a composition claim under examination (claim 12) and remains withdrawn. Claim 3 was amended to correct a typographical error. The amendments add no new matter; entry of the amendments is respectfully requested.

The grounds for rejection have been carefully considered, and the following remarks are offered in response. Reconsideration in view of these remarks is respectfully requested.

The Examiner indicated in the Office Action that the phrase "for enhancing an immune response" in claim 12 is considered an intended use and is not given patentable weight because the claim is drawn to a composition. In view of this position by the Office, that phrase has been canceled from claim 12, and its cancellation is believed to have no effect on the scope or patentability of the claim.

The Examiner has further taken the position that the phrase 'providing an enhanced immune response to the antigen than provided without the tryptanthrin compound adjuvant' in claim 12 is also given little patentable weight "since a composition and its properties are inseparable."

The Applicants traverse this position. The Examiner said that 'a composition and its properties are inseparable'. However, the phrase in claim 12 limits the composition in a substantive way, though it does so using a functional limitation. This phrase is part of a limitation on the composition: it describes compositions where the amount of the tryptanthrin compound is 'effective' to provide an enhanced immune response. Without this phrase, the claim reads on any composition having any amount of the tryptanthrin compound. Plainly, not all such compositions would be expected to do this; the presence of a single molecule of the tryptanthrin compound would not be expected to have a measurable effect on the immune response. However, the specification indicates that the compound has this effect when present 'in an effective amount' and it provides guidance for determining an amount that is effective; see, e.g., paragraph [0102]. It also provides guidance regarding amounts effective to achieve an effect, see e.g., paragraph [0103].

The limitation uses functional language to describe the effective amount, but that is perfectly proper where it would be clear to the person of ordinary skill in the art. MPEP 2173.05(g), citing as examples *In re Barr*, 444 F.2d 588, 170 USPQ 33 (CCPA 1971), and *In re Venezia*, 530 F.2d 956, 189 USPQ 149 (CCPA 1976). The claims encompass a class of compounds which will vary in their effects, and are suitable for administration to various subjects; it is understood and unavoidable that the 'effective amount' will vary, too. Thus a functional limitation, supported by explanations in the specification about how it is measured, is an appropriate way to describe the amount. Indeed, "an effective amount" is widely used and accepted as a suitable claim limitation in pharmaceutical practice. Here, the challenged language explains what the amount is "effective" for, and the specification provides guidance for identifying an effective amount, thus use of this functional language is appropriate, and it has been retained in the claim.

The Examiner has also taken the position that the phrase "enhances an immune response to the antigen and the immune response is the cellular production of one or more cytokines" in claim 15 is not given patentable weight for the same reason as above, "since a composition and its properties are inseparable."

The specification indicates that the immunogenicity-enhancing effect can be measured in different ways; increasing cytokine production is one of them. Again the Applicant has retained this phrase in the claim, and believes that it contributes to defining the composition, because it clarifies what effect is produced and how it is to be measured—by the cellular production of one or more cytokines.

The Examiner also stated that a composition and its properties are inseparable, so if the prior art teaches the composition, its properties are necessarily present. The Examiner cited *In re Spada*, saying "if the prior art teaches the identical chemical structure, the disclosed properties are necessarily present." However, that is inapplicable in this situation: the prior art **does NOT disclose these compositions**, so their properties are not present in the prior art, and their properties cannot be ignored in an obviousness analysis. The compounds of Formula I were at least partially known, so their properties were inherent in the prior art; but they were not known admixed with an antigen, so the property of enhancing the effect of an antigen is NOT inherently in the prior art. Because the combination as claimed was ***not*** previously known, its properties are not inherent in the prior art, and those properties should, indeed *must*, be considered in an obviousness analysis.

The only outstanding rejection (and the only rejection in the previous Office Action, also) is based on alleged obviousness; the compositions have thus been found novel over all cited art. Thus the properties of the compositions as claimed are new. This is very important to keep in mind, because the Unexpected Properties of a novel composition can make the composition patentable, even if each component of the composition was known, and even if there is a *prima facie* case for finding the combination of components to be obvious. See MPEP 716.02(a):

> A greater than expected result is an evidentiary factor pertinent to the legal conclusion of obviousness ... of the claims at issue." *In re Corkill*, 711 F.2d 1496, 226 USPQ 1005 (Fed. Cir. 1985). In *Corkhill*, the claimed combination showed an additive result when a diminished result would have been expected. This result was persuasive of nonobviousness even though the result was equal to that of one component alone. Evidence of a greater than expected result may also be shown by demonstrating an effect which is greater than the sum of each of the effects taken separately (i.e., demonstrating "synergism"). *Merck & Co. Inc. v. Biocraft Laboratories Inc.*, 874 F.2d 804, 10 USPQ2d 1843 (Fed. Cir.), *cert. denied*, 493 U.S. 975 (1989).

Indeed, in this case the composition reads on a mixture of materials at least some of which may have been previously known, but they had not been combined; and *in this case* the combination provides unexpected properties that the one of ordinary skill in the art would never have suspected prior to the time this invention was made, e.g., the immunogenic effect of an antigen is unexpectedly enhanced by a compound of Formula I. Those properties must be considered in an obviousness analysis.

Rejection Under 35 U.S.C. § 103

Claims 12-17 and 19 were rejected as allegedly obvious based on Baker et al. (US 5,441,955) in view of Colston et al. (US 7,122,195). According to the Examiner, Baker et al teach the tryptanthrin compounds of the specification as part of an antimicrobial composition, and teach that they can be administered 'with one or more other agents used in the treatment of pathogenic mycobacterial infections.' However, according to the Examiner, Baker fails to disclose a specific combination of the tryptanthrin compound (No. 1001) and an antigen disclosed in claim 14, which is allegedly cured by Colston:

> However, Colston et al. teach that recA mutant mycobacteria, particularly mutants of mycobacterial species which are members of Mycobacterium tuberculosis, are useful as vaccines for the treatment of a range of disorders, including tuberculosis (abstract)…Therefore it would have been prima facie obvious to a person of ordinary skill in the art, at the time the claimed invention was made, to combine the tryptanthrin compound (No. 1001) as disclosed by Baker et al. with the composition comprising antigens associated with tetanus or diphtheria disclosed by Colston et al…because (1) both Baker and Colston are analogous since both teach the treatment of pathogenic mycobacterial infections, for example tuberculosis; (2) Baker teaches that the tryptanthrin compound can be administered with an adjuvant or another agent used in the treatment of pathogenic mycobacterial infections; (3) Baker [sic: Colston?] teaches the use of antigens such as BCG in a vaccine against tuberculosis; (4) Colston teaches an antigen delivery system in the treatment of any disease, such as pathogenic infection, which is ameliorated by an immune response against a particular antigen; and (5) Colston specifically discloses suitable antigens, such as include [sic] viral, protozoal, tumour cell, bacterial, and fungal antigens, for example antigens from the Tetanustoxin and Diphteriatoxin.

> 'It is *prima facie* obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose... The idea of combining them flows logically from their having been individually taught in the prior art.' *In re Kerkhoven*, 626 F.2d 846, 850, 205 USPQ 1069, 1072 (CCPA 1980).

The Applicants traverse this rejection.

The Examiner cited BCG as one vaccine to be mixed with compounds of Formula I. **Exhibit A**, obtained from the internet at http://www.nlm.nih.gov/medlineplus/druginfo/medmaster/a682809.html describes how this material is to be used, and it specifically cautions the user to "tell your doctor and pharmacist what prescription and nonprescription medications you are taking, especially antibiotics..." This highlights something well known in the medical field: drug interactions are common. Because of this, one does not casually combine drugs merely because they are known in a general sense to be useful for treating a particular type of condition. In this case, the user of BCG is specifically advised to tell her doctor if she is using antibiotics, which suggests that drug interactions with antibiotics are a concern for a user of BCG. One reason for this is suggested by Colston, which states this about the efficacy of BCG: "The ability of BCT to survive for prolonged periods without causing progressive infection in immunocompetent individuals is an important componenet of its protective properties." Col. 2, lines 45-48.

In the present situation, the compounds of Formula I are said to be disclosed in Baker; Baker expressly describes its compounds as antibacterials, which are a type of antibiotic; moreover, its compounds kill mycobacteria. See Baker, col. 1, lines 7-14: "The present invention relates to new indolo[2,1-b]quinazoline-6,12-dione derivatives which are useful in killing mycobacteria, to antimicrobial compositions containing the compounds and to the use of the compounds and compositions, alone or in combination with other antimicrobial agents, in the treatment of pathogenic mycobacterial infections." The person of ordinary skill would have been aware that merely because an antibacterial of Baker could be used to treat conditions that could also be treated by vaccines of Colston, it would not be logical to combine the two, especially since BCG comprises a living mycobacterium that is essential to its function, and the compounds of Baker were described

as effective to kill mycobacteria: the combination would be expected to be ineffective, since the compound would be expected to kill the vaccine's active cells.

Even if a subject were to be *treated* with both substances, that does not render obvious a composition that *combines* the two components. Vaccines are typically administered once—see, e.g., **Exhibit A** ("When the vaccine is given to protect against TB, it usually is given only one time…") Antibacterials, on the other hand, are typically administered over a period of days and in many doses, and indeed it is standard procedure to require a patient to take a full series of dosages to reduce the likelihood of recurrence of the infection and of resistance development. It would thus NOT be appropriate to combine the two into a single composition: even if both are used to treat a single patient, the person of ordinary skill would NOT mix them in a single composition as claimed herein. The guidance on the usage of BCG is directly contrary to the Examiner's theory that 'treatment' with a vaccine and an antibacterial is equivalent to mixing the two into one composition. The general guidance to treat a TB patient with other drugs in Baker is exemplified by other small-molecule drugs; and even they are not said to be mixed with the compound of Formula I. A person of ordinary skill would certainly not have been motivated by that general comment to *mix together* the antibacterial of Baker and the vaccine components of Colston.

Administering BCG along with an antibacterial like the compounds of Formula I would not be logical, because the antibacterial would be expected to harm the bacteria in the vaccine or in the treated patient. More emphatically, combining the antibacterial with the vaccine, where the antibacterial is present in an amount intended to be useful to treat a patient, would certainly expose the BCG cells to a dose of antibacterial that would be expected to be fatal to the cells and thus destroy the usefulness of the vaccine. As stated in Baker, "The present invention relates to new indolo[2,1-b]quinazoline-6,12-dione derivatives which are useful in killing mycobacteria, to antimicrobial compositions containing the compounds and to the use of the compounds and compositions, alone or in combination with other antimicrobial agents, in the treatment of pathogenic mycobacterial infections." This is a clear, if implicit, *teaching away* from mixing BCG with an antibacterial compound: the person of ordinary skill would recognize that the combination would not be expected to function. The person of ordinary skill would *Not* have been motivated to

combine the two, and would not have had a 'reasonable expectation' that the combination would work.

Neither Baker nor Colston discloses or suggests a composition that includes an antibacterial such as the Baker compounds mixed with a vaccine component or antigen. Baker identifies several examples of other 'treatments' to combine with its compound, but they are all small-molecule drugs: none of them is a vaccine or operates like a vaccine. The differences between a small molecule drug and a vaccine, including the differences in the way they work and the differences in the way they are administered, are well known to a person of ordinary skill. The Examiner has not identified even one reference where a vaccine and an antibacterial were mixed. Thus, even if both were used to treat a particular patient, the claimed mixture in a single composition is NOT *prima facie* obvious, because *it would not have been made by a person of ordinary skill in the art*, and the Examiner has provided nothing to suggest such a mixture.

The Examiner cited *In re Kerkhoven*, saying "It is *prima facie* obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose…The idea of combining them flows logically from their having been individually taught in the prior art." But in *Kerkhoven*, the court also said combining the compositions would "require no more than the mixing together of two conventional spray-dried detergents." That is an entirely different situation from this: the compositions in this case are not merely two conventional cleaning agents that one might casually mix together. Here, one of the compositions is an antibacterial compound, which acts by killing a bacterium, according to Baker. The other is a vaccine composition that acts by a completely different mechanism, eliciting an immune response. Mixing them together is quite unlikely mixing two types of detergents, which is all that was at issue in *Kerkhoven*. The person of ordinary skill in the art knows this; and even if 'the idea of combining them flows…', the person of ordinary skill knows why that would be inappropriate (drug interactions, different administration methods and schedules, etc.) and would not have pursued the idea.

The Examiner has the burden to show that a person of ordinary skill would have been motivated to make the particular combination claimed, with a reasonable expectation of success. Where combining things together is more complicated than mixing two detergents—like here, where such a combination implicates both the effectiveness of the components and the health of a patient—the person of ordinary skill factors in his/her knowledge about the components and does not act rashly. In this case, the person of ordinary skill in the art is a physician or similarly skilled person who understand the difference between an antibacterial and a vaccine. The person of ordinary skill would recognize that these two materials require different formulations, different administration schedules, and different storage conditions. The person of ordinary skill understands that they have different effects, serve different purposes, and are suitable for use in different patients. *Kerkhoven* is insufficient basis to allege that a person of ordinary skill in the art would have found it obvious to mix the antibacterial of Baker with the vaccine component of Colston. **The person of ordinary skill certainly would not have relied upon *Kerkhoven* or its reasoning.** That case refers to two cleaning agents used 'for the same purpose,' where the combination was to be used for "the very same purpose." But the terms 'same purpose' and 'very same purpose' must be understood in the context of the *Kerkhoven* fact pattern. The statements in *Kerkhoven* should not be abused by applying them to a situation where the two compositions act by entirely different mechanisms, and where the mixture of two compositions is to be administered to a patient whose health depends upon the decision. More importantly, in this case, the person of ordinary skill knows there are risks to a patient when combining materials, and also knows clear reasons not to combine the compositions.

Even if a physician were motivated to use both the antibacterial compound of Baker and the vaccine composition of Colston together, they would be used to *treat* one subject. The person of ordinary skill would not have combined the two into a single composition. There is no need to do so, and there are many reasons not to do so. The particular mixture of the claims is not rendered obvious by the very general suggestion to use Baker's compounds with 'other treatments;' especially where the exemplary 'other treatments' that Baker mentioned do not suggest using its compounds with vaccines—indeed, in the Abstract Baker mentions these other substances and refers to them as 'other antimicrobial agents.' The Colston materials that the Examiner proposes

adding to Baker's compounds are vaccine components that have no antimicrobial activity. Moreover, the general comment in Baker certainly does not say that such treatment requires mixing its compounds with other materials, even if both are used to treat a patient. And the analogy of this situation to *Kerkhoven* is inappropriate because of the vastly different fact patterns involved. The person of ordinary skill would certainly see the facts in this case to be quite different from those in *Kerkhoven*; and the obviousness analysis must be assessed from the perspective of a person of ordinary skill in the art.

In addition, Baker's compounds are antibacterials. Colston's compositions are actually not just antigens: the title of Colston's patent is "Mutant Mycobacteria for Use in Therapy." Its text says this, "A mycobacterial cell of the present invention persists in a host immunized therewith. The presence of the recA mutation does not affect the ability of the cell to survive in the host and the cell is able to persist in tissue without causing progressive infection in an immunocompetent host." Colston, col. 3, lines 49-53. This is what the passage from Colston cited in the Office Action says (col. 4, line 46 to col. 5, line14):

> A cell of a mycobacterium of the *M. tuberc*ulosis complex as described herein, for example a *Mycobacterium bovis* BCG or *Mycobacterium tuberculosis* cell, may be used in a method of treatment of the human or animal body, for example a method of therapeutic treatment.
>
> A *M. tuberculosis* complex cell of the present invention may further comprise a gene encoding a polypeptide which is a non-mycobacterial or foreign antigen. Expression of such an antigen in an *M. tuberculosis* complex cell, for example, *an M. bovis* BCG cell allows the generation of an immune response in a vaccinated individual against the non-mycobacterial antigen. The cell may therefore be used as an antigen delivery system in the treatment of any disease, such as a pathogenic infection, which is ameliorated by an immune response against a particular antigen.
>
> The improved genetic stability of mycobacterial cells of the present invention provides for the improved retention of the non-mycobacterial gene and therefore a more effective immune response.
>
> Suitable antigens include viral, protozoal, tumour cell, bacterial and fungal antigens. For example, an antigen from *H. pylori*, Measles virus (Fennelly G. J. et al (1995) *J. Infect. Dis.* 172: 698 705), Mumps virus, Rubeola virus (e.g. OspA: Stover, C. K. et al

(1993) *J. Exp. Med.* 178: 197 209), *B. burgdorferi* (e.g. protein A: Langermann et al (1994) *Nature* 372: 552 555), Herpesvirus, Papillomavirus, Tetanustoxin, Diphtheriatoxin, *Pneumococcus* spp (e.g. Surface protein A: Langermann et al (1994) J. Exp. Med. 180: 2277 2286) tumour cells, Leishmania (e.g. surface proteinase gp63: Connell N. et al (1993) *Proc. Natl. Acad. Sci. USA*. 90: 11473 11477) or HIV (or SIV: Yasutomi Y. et al (1993) *J. Immunol*. 150: 3101 3107) may be used. Such an antigen may be useful in the treatment of ulcers, measles, mumps, rubeola, Lyme disease, herpes, cancer, tetanus, diphtheria, cancer, Leishmaniasis or AIDS respectively.

Colston is a patent about using certain antigens with a living cell that is adapted to enhance an immune response to the antigen. The passage about antigens does not describe using an antigen alone to treat an infection: it describes using the antigens along with a living cell. That living cell is an essential part of what Colston says is effective for treating an infection. The person of ordinary skill would have understood this passage of Colston to describe using an antigen along with a particular type of cell, which is needed to elicit a strong immune response. However, the cells that Colston uses are a mycobacterium, such as *M. tuberculosis*; and the compounds of Baker are useful to treat infections *because they kill bacteria*. Since Colston says its compositions are effective because they use living bacteria, and Baker's compounds are effective because they *kill* bacteria, it would certainly NOT be appropriate to mix those cells with the compounds from Baker. One of ordinary skill would NOT have combined the compositions of Baker and Colston, because the compound of Baker would be expected to kill the cell of Colston. Indeed, in view of the antibacterial activity of the Baker compounds and the stated need to have the cells of the Colston vaccine survive in a host, one would not have used the antibacterial in the same subject while or soon after the vaccine was administered to the subject. Moreover, one would not be motivated to *remove* the cell from the Colston vaccine composition, because Colston relies upon its cell to enhance the antigen's ability to elicit an immune response: one would not remove the cell because it defeats the purpose of the Colston invention, and without the cell to activate the antigen, one would not reasonably expect the Colston composition to work. And one could not have known from Baker or Colston that the cell might not be necessary when a compound of Formula I is present; because it was not known before the present invention that the compound of Formula I can serve to enhance the antigenicity of an antigen.

Moreover, Exhibit B is a reference that discusses interactions between vaccine compositions and antibiotics. It demonstrates that vaccines are often antagonized (made less effective) by antibiotics, so it was not predictable that mixing an antibacterial compound of Baker with an antigenic composition would work, prior to the present invention. In the particular case of a BCG vaccine, or the mycobacterial compositions of Colston, a reason for this is apparent.

In addition, the combination of Colston with Baker is improper because the addition of Baker's compound to Colston's vaccine compositions would render the Colston product ineffective. Combining reference teachings for an obviousness rejection is improper if the combination would render the prior art inoperable: MPEP 2143.01(V) ("If proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984)" (emphasis added)). In this case, the compound from Baker is expected to kill the cell required in Colston's vaccine composition, thus rendering the vaccine inoperable and 'unsatisfactory for its intended purpose.' There is thus not a proper motivation to combine these teachings to produce one composition.

Finally, even if a *prima facie* case of obviousness were established, which it has not for all the reasons presented above, it would be overcome by evidence of an unexpected result. The Examiner refused to consider such evidence before, stating that the effect is not unexpected "since tryptanthrin is a known compound." This is an incorrect and erroneous analysis: the effect of combining these compositions as claimed is *NOT inherent in any prior art*, because the composition is not in the prior art. This composition is novel; the Examiner arrives at it by combining prior art teachings, not by finding this composition in the prior art. There is no prior art composition having this effect, and no evidence in the prior art that a composition might have this effect.

The present invention demonstrates essentially a synergistic effect obtained by using the compound of Formula I to enhance the antigenicity of an antigen. Before the present invention, at most one might have expected using the Colston vaccine components with the Baker compounds to produce the same antigenic effect as the vaccine components alone, and the same antibacterial effect

as the compound of Formula I alone. (And of course for reasons described above, one would not have *combined* them even if used to treat the same subject.) As described in the specification, that is not the result: unexpectedly, the compound of Formula I enhances the immunogenic effect of an antigen mixed with it—which would not have been expected before the present invention was made.

The Examiner's rationale for combining Baker's compounds with the Colston antigens is based on using each of them for its separate effects as a treatment for an infection. As demonstrated above, this would not be expected to work. For the numerous reasons discussed above, a person of ordinary skill would not have combined antigenic compositions of Colston with the antibacterial compound of Baker. If one thought to combine them, one would be informed by knowledge in the art, *e.g.,* Exhibit B, that the result would not be predictable, because the compounds of Formula I are antibacterials, and it is known that antibiotics can reduce the effectiveness of an antigen. In the combination of references the Examiner discusses, this is particularly significant, because the Colston composition, like BCG, relies upon a living bacterium to provide an antigenic effect, and the Baker compounds are said to kill bacteria. And even if they two were combined, their effect would have been expected to be additive at most; there was no reason to expect it to be better than that. Yet, as taught in the specification, the compositions provide a greater antigenic effect than one would have expected, because the compounds of Formula I enhance the immunogenic effect of an antigen. This evidence of an unexpected effect is evidence of nonobviousness, which must be considered in the obviousness analysis, and overcomes any allegations of obviousness presented.

In view of the evidence above, one of ordinary skill in the art would not have been motivated to combine the compounds of Baker with the antigenic compositions of Colston, because:

The two do not serve the 'very same purpose';

The two require different formulations and administration schedules; and

Nothing in either reference suggests that an antibacterial can be usefully combined with a vaccine. And other knowledge in the art, as well as the fact that the products of Baker and

Colston are simply incompatible with each other, provides strong teaching away from combining antibacterials of Baker with such vaccine compositions of Colston.

If one were motivated to combine the two compositions, one would not have had a reasonable expectation of success, because:

Exhibit B demonstrates that antibiotics often interact adversely with vaccines;

And the compound of Baker is expected to kill the cell that is used by Colston to make its antigen an effective treatment.

And finally, even if a *prima facie* case for obviousness were established, it is overcome by the evidence of record, recognized by the Examiner: the Valiante declaration shows that the combination claimed produces an enhanced antigenic effect that could not have been expected before the invention was made.

In view of these facts and the supporting evidence provided herewith, the Applicants respectfully request the withdrawal of the obviousness rejection, and allowance of the pending claims.

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 223002107100. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

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Respectfully submitted,

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EXHIBIT A

Drug Information: Bacillus Calmette-Guerin (BCG) Vaccine

URL of this page: http://www.nlm.nih.gov/medlineplus/druginfo/medmaster/a682809.html

(ba sil' us kal' met gay rayn')

WHY is this medicine prescribed?

BCG vaccine provides immunity or protection against tuberculosis (TB). The vaccine may be given to persons at high risk of developing TB. It is also used to treat bladder tumors or bladder cancer.

This medication is sometimes prescribed for other uses; ask your doctor or pharmacist for more information.

HOW should this medicine be used?

Your doctor or a health care provider will administer this medicine. When used to protect against TB, it is injected into the skin. Keep the vaccination area dry for 24 hours after receiving the vaccine, and keep the area clean until you cannot tell the vaccination area from the skin around it.

When used for bladder cancer, the medicine flows into your bladder through a tube or catheter. Avoid drinking fluids for 4 hours before your treatment. You should empty your bladder before treatment. During the first hour after the medication is infused, you will lie on your stomach, back, and sides for 15 minutes each. Then you will stand, but you should keep the medication in your bladder for another hour. If you cannot keep the medication in your bladder for the entire 2 hours, tell your health care provider. At the end of 2 hours you will empty your bladder in a seated manner for safety reasons. Your urine should be disinfected for 6 hours after the medication is administered. Pour a similar amount of undiluted bleach in the toilet after you urinate. Let it stand for 15 minutes before flushing.

Various dosing schedules may be used. Your doctor will schedule your treatment. Ask your doctor to explain any directions you do not understand.

When the vaccine is given to protect against TB, it usually is given only one time but may be repeated if there is not a good response in 2-3 months. Response is measured by a TB skin test.

What SPECIAL PRECAUTIONS should I follow?

Before receiving BCG vaccine,

- tell your doctor and pharmacist if you are allergic to BCG vaccine or any other drugs.
- tell your doctor and pharmacist what prescription and nonprescription medications you are taking, especially antibiotics, cancer chemotherapy agents, steroids, tuberculosis medications, and vitamins.
- tell your doctor if you have had a recent smallpox vaccination or if you have had a positive TB test.
- tell your doctor if you have an immune disorder, cancer, fever, an infection, or an area of severe burns on your body.
- tell your doctor if you are pregnant, plan to become pregnant, or are breast-feeding. If you become pregnant while taking BCG vaccine, call your doctor immediately.

What SIDE EFFECTS can this medicine cause?

BCG vaccine may cause side effects. Tell your doctor if any of these symptoms are severe or do not go away:

- swollen lymph nodes
- small red areas at the site of injection. (These usually appear 10-14 days after injection and slowly decrease in size. They should disappear after about 6 months.)
- fever
- blood in the urine
- frequent or painful urination
- upset stomach
- vomiting

If you experience any of the following symptoms, call your doctor immediately:

- severe skin rash
- difficulty breathing or swallowing
- wheezing

What should I do in case of OVERDOSE?

In case of overdose, call your local poison control center at 1-800-222-1222. If the victim has collapsed or is not breathing, call local emergency services at 911.

What OTHER INFORMATION should I know?

Keep all appointments with your doctor and the laboratory.

Brand name(s):

- TheraCys® BCG
- TICE® BCG

Other name(s):

- BCG live
- BCG vaccine

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Antibiotics Modulate Vaccine-Induced Humoral Immune Response

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The effects of antibiotics on the antigen-specific humoral immune response are not known. Macrolides, tetracyclines, and beta-lactams are commonly prescribed antibiotics. The first two are known to have immunomodulatory activities. The effects of clarithromycin, doxycycline, and ampicillin on the primary and secondary antibody responses to tetanus toxoid, a pneumococcal polysaccharide vaccine, a hepatitis B virus surface antigen (HBsAg) vaccine, and live attenuated *Salmonella typhi* (Ty21a) were investigated using a mouse model. For the mice receiving the tetanus toxoid, the immunoglobulin M (IgM) level of the clarithromycin group at day 7 was significantly lower than the corresponding antibody level of the normal saline (NS) group. For the mice receiving the pneumococcal polysaccharide vaccine, the total antibody and IgM levels of the clarithromycin group and the IgM level of the doxycycline group at day 7 were significantly lower than the corresponding antibody levels of the ampicillin and NS groups. For the mice receiving the HBsAg vaccine, the IgM level of the doxycycline group at day 7 was significantly lower than the corresponding antibody levels of the clarithromycin and NS groups, while the IgM level of the clarithromycin group at day 28 was significantly lower than the corresponding antibody levels of the doxycycline, ampicillin, and NS groups. For the mice receiving all three vaccines, there were no statistically significant differences between any of the antibody levels of the ampicillin group and the corresponding antibody levels of the NS group. For the mice receiving Ty21a, the total antibody levels of the ampicillin group at days 7 and 21 were significantly higher than the corresponding antibody levels of the NS group. Moreover, the IgM levels of the clarithromycin, doxycycline, and ampicillin groups at days 7 and 21 were significantly higher than the corresponding antibody levels of the NS group. Furthermore, the total antibody level of the ampicillin group at day 21 was significantly higher than the corresponding antibody level of the doxycycline group. For all four vaccines, there were no statistically significant differences among the serum levels of interleukin-10 and gamma interferon for the mice treated with the various antibiotics. We conclude that clarithromycin and doxycycline, but not ampicillin, suppress the antibody responses of mice to T-cell-dependent and T-cell-independent antigens, whereas all three antibiotics enhance the antibody response to live attenuated mucosal bacterial vaccines.

Antibiotics are well-known to have effects on the immune system, as shown by in vitro, ex vivo, and in vivo animal experiments and clinical studies. Regarding macrophage-monocyte functions, in vitro experiments have shown that macrolides stimulate phagocytic chemotaxis (4), promote monocyte-to-macrophage differentiation (11), and increase the killing capacity of macrophages (6); tetracyclines inhibit phagocytic chemotaxis and granuloma formation (25). As for cytokines, macrolides inhibit interleukin-1 (IL-1) production by murine peritoneal macrophages (22) and suppress IL-2 production induced by mitogen-stimulated T cells (15), while tetracyclines inhibit IL-1 and tumor necrosis factor alpha (TNF-α) production by human macrophages (19). In regard to lymphocytes, macrolides suppress mixed lymphocyte proliferation and the proliferative response of human peripheral blood mononuclear cells stimulated by polyclonal T-cell mitogens (15). Additionally, tetracyclines can protect mice from lethal endotoxemia (13), and we have recently shown that clarithromycin attenuates the surgical-trauma-induced inflammatory response in guinea pigs (26) and cyclophosphamide-induced mucositis in mice (27). In clinical studies, it has been shown that erythromycin has an anti-inflammatory effect on patients with diffuse panbronchiolitis (17). Despite these findings, most of the experimental data to date relate to how antibiotics affect the innate immune response, cytokine levels, or nonspecific monocyte or lymphocyte proliferation. It has never been shown quantitatively how these antibiotics affect the effector arms of adaptive immunity, namely specific-antigen-induced antibody production and specific-antigen-induced lymphocyte proliferation or epitope-specific cytotoxic T-cell responses. The only study of antibody production and allograft rejection was not antigen specific (2).

Tetanus toxoid, pneumococcal polysaccharide vaccine, hepatitis B virus surface antigen (HBsAg) vaccine, and live attenuated *Salmonella typhi* are the prototypes of T-cell-dependent inactivated toxin, T-cell-independent polysaccharide, recombinant protein, and live attenuated vaccines, respectively. Their protective efficacies are often associated with the induction of antibody production in the host (3, 8, 10, 16, 21, 24). Since antibiotics of the macrolide, tetracycline, and penicillin groups are commonly prescribed and some of them have known effects on the immune system, but minor ailments such as upper respiratory tract infections may require antibiotic treatment and such treatment is not a known contraindication to vaccination, it is important to know whether antibiotics have any effects on the efficacy of immunization. In these experiments, we investigated the effect of clarithromycin (a commonly pre-

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scribed macrolide), doxycycline (a commonly prescribed tetracycline), and ampicillin (a commonly prescribed penicillin without a known effect on the immune system) on antibody production after tetanus toxoid, pneumococcal polysaccharide vaccine, HBsAg vaccine, and live attenuated *S. typhi* (Ty21a) administration to mice.

MATERIALS AND METHODS

Animals. Female BALB/c mice (18 to 22 g) were used in all experiments. They were housed in cages, each containing 10 mice, under standard conditions with regulated day length, temperature, and humidity, and they were given pelleted food and tap water ad libitum.

Immunization. On day zero, groups of 40 mice were immunized subcutaneously with tetanus toxoid with alum adjuvant (Berna, Bern, Switzerland; 2 limit flocculations (Lf) per mouse), subcutaneously with a pneumococcal polysaccharide vaccine (Pneumovax 23; Merck, Rahway, N.J.; 0.5 μg of each polysaccharide antigen per mouse), intraperitoneally with an HBsAg vaccine with alum adjuvant (H-B-VAX II; MSD, Whitehouse Station, N.J.; 0.5 μg per mouse), or intraperitoneally with live attenuated *S. typhi* (Ty21a; Berna; 10^9 CFU per mouse) transformed with pBR322 (Amersham Pharmacia Biotech, Piscataway, N.J.) by electroporation (so as to make it ampicillin and doxycycline resistant [it is intrinsically resistant to clarithromycin]). On day 21, the same amount of tetanus toxoid, pneumococcal polysaccharide vaccine, or HBsAg vaccine was given to each member of the corresponding group of mice as a booster dose.

Administration of antibiotics. Clarithromycin (50 mg/kg), doxycycline (1.5 mg/kg), ampicillin (20 mg/kg), or normal saline (NS) (0.25 ml) was administered intraperitoneally to the 10 mice of each group daily from 1 day prior to immunization (day −1) to day 27 postimmunization for the tetanus toxoid, pneumococcal polysaccharide vaccine, and HBsAg groups or to day 20 postimmunization for the Ty21a groups.

Measurement of antibody response. The mice were bled on days −1, 7, 21, and 28 for the tetanus toxoid, pneumococcal polysaccharide vaccine, and HBsAg groups and on days −1, 7, and 21 for the Ty21a groups. On days −1, 7, and 21, blood was taken just prior to administration of antibiotics. The blood was centrifuged at 2,700 × *g* for 20 min, and the supernatant (serum) was aliquoted and stored at −70°C until antibody measurements were performed.

Nunc-Immuno plates (Naige Nunc International, Roskilde, Denmark) were used in all enzyme-linked immunosorbant assay (ELISA) experiments for measurement of antibody levels against tetanus toxoid, pneumococcal polysaccharide, and lipopolysaccharide of *S. typhi*. Each well was coated with 100 μl of diluted antigen (50 μl of tetanus toxoid in 50 μl of 0.05 M carbonate-bicarbonate buffer [pH 9.6], 0.1 μl of pneumococcal polysaccharide in 99.9 μl of phosphate-buffered saline [PBS], or 4 μg lipopolysaccharide of *S. typhi* in 0.05 M carbonate-bicarbonate buffer [pH 9.6]), and the plates were incubated at 4°C overnight. After the plates were washed with PBS–0.05% Tween 20 (washing buffer) twice, 200 μl of PBS–5% bovine serum albumin (BSA) (blocking buffer) was added to each well; the plates were then incubated at 37°C for 2 h. After the ELISA plates were washed with washing buffer three times, mouse sera (diluted with PBS–2% BSA) were added to them. For measurement of antibody levels against HBsAg, mouse sera (diluted with PBS–2% BSA) were added to ELISA plates precoated with HBsAg (Biokit, Barcelona, Spain). The plates were incubated at 37°C for 1 h. After the plates were washed with washing buffer three times, 100 μl of peroxidase-conjugated goat anti-mouse antibody (Serotect, Kidlington, United Kingdom), diluted with PBS–2% BSA according to the manufacturer's instructions, was added to each well; the plates were then incubated at 37°C for 30 min (tetanus toxoid, pneumococcal polysaccharide, and HBsAg) or 1 h (Ty21a). Immunoglobulin M (IgM) and total antibody levels were assayed to assess the primary and secondary immune responses, while IgG1 and IgG2a were measured to determine whether the humoral response had a more Th2-like or Th1-like pattern, respectively. After the plates were again washed with washing buffer three times, 100 μl of *ortho*-phenylenediamine (OPD) substrate (prepared by diluting 2 mg of OPD [Cadbiochem, La Jolla, Calif.] in 2.5 ml of 50 mM citric acid [pH 5] with 2.5 μl of 30% H_2O_2) was added to each well; the plates were then incubated at room temperature for 30 min. A 100-μl aliquot of 1 M H_2SO_4 was added to each well, and the absorbance of each well was measured at 492 nm, using OPD buffer as a blank. Each sample was tested in duplicate, and the mean absorbance for each serum was calculated. All ELISAs were optimized so that there was a linear relationship between the optical density and the amount of antibody present in the serum at the serum dilution for the corresponding type of antibody measured. The serum antibody level of a particular mouse on a particular day was defined as the absorbance obtained from the serum on that day minus that of the same mouse on day −1. Control experiments were performed by adding ampicillin, clarithromycin, or doxycycline to serum samples so as to exclude the possibility of antibiotics interfering with the ELISA.

Measurement of serum levels of IL-10 and IFN-γ. Serum IL-10 and gamma interferon (IFN-γ) were measured by using commercial kits (Amersham Pharmacia, Little Chalfont, United Kingdom) to determine whether the immune response was more Th2 or Th1 like, respectively. Briefly, 50 μl of serum from each sample was added to the wells of ELISA plates precoated with monoclonal antibodies against IL-10 or IFN-γ. The plates were incubated at 25°C for 3 and 2 h, respectively. For IL-10, after the plates were washed with washing buffer three times, 50 μl of biotinylated antibody against IL-10 was added to each well, and the plates were then incubated at 25°C for 1 h. After the plates were again washed with washing buffer three times, 100 μl of streptavidin-horseradish peroxidase conjugate was added to each well prior to incubation at 25°C for 30 min. For IFN-γ, 100 μl of horseradish peroxidase-conjugated antibody against IFN-γ was added to each well. After the plates were washed with washing buffer three times, 100 μl of 3,3′-5,5′-tetramethylbenzidine substrate was added to each well, and the plates were incubated at room temperature for 30 min. Then 100 μl of 1 M H_2SO_4 was added per well, and the absorbance of each well was measured at 450 nm. The IL-10 and IFN-γ concentrations of individual samples were calculated by using standard curves prepared by performing the ELISA with known concentrations of the cytokines. The serum IL-10 or IFN-γ level for a particular mouse on a particular day is defined as the concentration of the cytokine on that day minus that of the same mouse on day −1.

Statistical analysis. Comparisons of the antibody and cytokine levels of mice in the clarithromycin, doxycycline, ampicillin, and NS groups receiving tetanus toxoid, pneumococcal polysaccharide vaccine, recombinant HBsAg vaccine, or Ty21a transformed with pBR322 were made by using Tukey's honestly significant difference test. A $P < 0.05$ is regarded as statistically significant.

RESULTS

The antibody levels at days 7, 21, and 28 after subcutaneous tetanus toxoid, subcutaneous pneumococcal polysaccharide vaccine, or intraperitoneal HBsAg vaccine administration to mice treated with clarithromycin, doxycycline, ampicillin, or NS are shown in Tables 1, 2, and 3, respectively. No effect of chemical interference of antibiotics on the ELISA was found, and there were no statistically significant differences among the antibody levels in the various groups of mice at day −1. For the mice receiving tetanus toxoid, the IgM level of the clarithromycin group at day 7 was significantly lower than the corresponding antibody level of the NS group. For the mice receiving the pneumococcal polysaccharide vaccine, the total antibody and IgM levels of the clarithromycin group and the IgM level of the doxycycline group at day 7 were significantly lower than the corresponding antibody levels of the ampicillin and NS groups. For the mice receiving the HBsAg vaccine, the IgM level of the doxycycline group at day 7 was significantly lower than the corresponding antibody levels of the clarithromycin and NS groups, while the IgM level of the clarithromycin group at day 28 was significantly lower than the corresponding antibody levels of the doxycycline, ampicillin, and NS groups. For the mice receiving all three of the vaccines, there were no statistically significant differences between the antibody levels of the ampicillin group and the corresponding antibody levels of the NS group.

The antibody levels at days 7 and 21 after intraperitoneal Ty21a administration to mice treated with clarithromycin, doxycycline, ampicillin, or NS are shown in Table 4. There were no statistically significant differences among the antibody levels in the various groups of mice at day −1. The total antibody levels of the ampicillin group at days 7 and 21 were significantly higher than the corresponding antibody levels of the NS group. Moreover, the IgM levels of the clarithromycin, doxycycline, and ampicillin groups at days 7 and 21 were significantly higher than the corresponding antibody levels of the NS group. Furthermore, the total antibody level of the ampicillin group at day 21 was significantly higher than the corresponding antibody level of the doxycycline group.

The serum IL-10 and IFN-γ levels of the mice administered the various vaccines and antibiotics are shown in Tables 5 and 6, respectively. For all four vaccines, there were no statistically significant differences among the IL-10 and IFN-γ levels of the mice administered the various antibiotics.

TABLE 1. Total antibody and antibody subtype levels at days 7, 21, and 28 after subcutaneous tetanus toxoid administration to mice treated with clarithromycin, doxycycline, ampicillin, or NS

Day postvaccination	Antibody subtype	Serum dilution	Antibody level (A_{492})					
			Mean for group treated with:				SEM (pooled)	Critical value
			Clarithromycin ($n = 10$)	Doxycycline ($n = 10$)	Ampicillin ($n = 10$)	NS ($n = 10$)		
7	Total	1:500	0.150	0.122	0.152	0.164	0.015	0.058
	IgM	1:100	0.417[a]	0.554	0.659	0.696[a]	0.065	0.247
	IgG1	1:50	0.265	0.216	0.186	0.173	0.048	0.182
	IgG2a	1:50	0.014	0.009	0.010	0.012	0.002	0.009
21	Total	1:5,000	0.484	0.486	0.480	0.472	0.044	0.168
	IgM	1:100	0.122	0.181	0.156	0.130	0.023	0.088
	IgG1	1:500	0.382	0.354	0.364	0.355	0.019	0.071
	IgG2a	1:50	0.020	0.040	0.032	0.023	0.007	0.027
28	Total	1:50,000	0.200	0.174	0.185	0.158	0.016	0.060
	IgM	1:100	0.191	0.207	0.196	0.151	0.024	0.091
	IgG1	1:5,000	0.286	0.217	0.235	0.243	0.025	0.096
	IgG2a	1:50	0.105	0.293	0.200	0.133	0.057	0.217

[a] The difference is statistically significant compared to the mean with the same superscript.

DISCUSSION

This is the first study undertaken to show the effects of antibiotics on the B-cell response induced by specific antigens in a series of common vaccines. These vaccines were chosen because they represent prototypes of T-cell-dependent inactivated toxin, T-cell-independent polysaccharide, recombinant protein, and live attenuated vaccines against bacteria and viruses; clarithromycin, doxycycline, and ampicillin were chosen because they are commonly prescribed for minor ailments such as upper respiratory tract infection and acne vulgaris, and doxycycline and clarithromycin are known to have immunomodulating activities.

It has been known for a long time that antibiotics have various effects on the immune system (18). A number of groups have reported immunomodulatory effects of the macrolides and tetracyclines in vitro. The macrolides roxithromycin and erythromycin enhanced the phagocytosis of ^{3}H-labelled *Staphylococcus aureus* by human macrophages (4) and increased the killing capacity for human macrophage-ingested live *Staphylococcus aureus* (6). Clarithromycin significantly inhibited IL production by murine peritoneal macrophages (22). Erythromycin significantly increased the number of adherent human macrophages derived from monocytes after 7 days of culture (11). At concentrations of 40 to 200 μg/ml, midecamycin, josamycin, and clarithromycin suppressed the proliferative response of human peripheral blood mononuclear cells stimulated by polyclonal T-cell mitogens, and they also suppressed IL-2 production induced by mitogen-stimulated T cells at concentrations between 1.6 and 40 μg/ml (15). The combination of erythromycin and granulocyte-macrophage colony-stimulating factor and macrophage colony-stimulating factor additively and synergistically increased the number of monocyte-derived macrophages (11). The expression of surface antigen CD71, a macrophage activation marker, was increased when human macrophages were cultured in the presence of erythromycin (11). Recently, it was also reported that erythromycin ameliorated some chronic inflammatory processes of the respiratory tract, such as diffuse panbronchiolitis (17) and bronchial asthma (14), irrespective of its antibacterial properties. In one study of patients with panbronchiolitis, it was shown that erythromycin improved respiratory function and arterial blood gas tension irrespective of the presence of *Pseudomonas aerugi-*

TABLE 2. Total antibody and antibody subtype levels at days 7, 21, and 28 after subcutaneous pneumococcal polysaccharide vaccine administration to mice treated with clarithromycin, doxycycline, ampicillin, or NS

Day postvaccination	Antibody subtype	Serum dilution	Antibody level (A_{492})					
			Mean for group treated with:				SEM (pooled)	Critical value
			Clarithromycin ($n = 10$)	Doxycycline ($n = 10$)	Ampicillin ($n = 10$)	NS ($n = 10$)		
7	Total	1:1,000	0.149[a,b]	0.217	0.281[a]	0.283[b]	0.031	0.118
	IgM	1:100	0.671[c,d]	0.673[c,f]	0.812[e,g]	0.807[d,f]	0.019	0.073
	IgG1	1:50	0.017	0.014	0.016	0.011	0.002	0.009
	IgG2a	1:50	0.012	0.011	0.010	0.010	0.001	0.004
21	Total	1:1,000	0.343	0.361	0.345	0.347	0.029	0.110
	IgM	1:1,000	0.442	0.477	0.452	0.430	0.020	0.077
	IgG1	1:50	0.017	0.011	0.016	0.019	0.004	0.014
	IgG2a	1:50	0.018	0.015	0.018	0.018	0.004	0.015
28	Total	1:1,000	0.277	0.298	0.284	0.277	0.034	0.128
	IgM	1:1,000	0.214	0.312	0.234	0.246	0.027	0.102
	IgG1	1:50	0.016	0.012	0.019	0.027	0.007	0.025
	IgG2a	1:50	0.020	0.031	0.022	0.021	0.009	0.036

[a–f] The difference is statistically significant compared to the mean with the same superscript.

TABLE 3. Total antibody and antibody subtype levels at days 7, 21, and 28 after intraperitoneal HBsAg vaccine administration to mice treated with clarithromycin, doxycycline, ampicillin, or NS

Day postvaccination	Antibody subtype	Serum dilution	Antibody level (A_{492})					
			Mean for group treated with:				SEM (pooled)	Critical value
			Clarithromycin ($n = 10$)	Doxycycline ($n = 10$)	Ampicillin ($n = 10$)	NS ($n = 10$)		
7	Total	1:100	0.203	0.212	0.200	0.193	0.016	0.060
	IgM	1:100	0.357[a]	0.103[a,b]	0.201	0.322[b]	0.029	0.210
	IgG1	1:50	0.027	0.026	0.026	0.027	0.002	0.007
	IgG2a	1:50	0.022	0.020	0.020	0.021	0.002	0.008
21	Total	1:100	0.293	0.466	0.506	0.721	0.143	0.543
	IgM	1:100	0.382	0.598	0.502	0.560	0.064	0.245
	IgG1	1:50	0.049	0.069	0.123	0.222	0.060	0.229
	IgG2a	1:50	0.060	0.039	0.099	0.162	0.045	0.172
28	Total	1:1,000	0.440	0.631	0.789	0.785	0.106	0.405
	IgM	1:100	0.379[c,d,e]	0.602[c]	0.565[d]	0.574[e]	0.041	0.158
	IgG1	1:50	0.335	0.710	1.056	1.130	0.222	0.845
	IgG2a	1:50	0.464	0.339	0.565	0.626	0.140	0.533

[a–e] The difference is statistically significant compared to the mean with the same superscript.

nosa in the sputum (7). As for the tetracyclines, tetracycline, doxycycline, and minocycline inhibited granuloma formation in vitro in a dose-dependent manner at concentrations between 10^{-4} and 10^{-6} mol/liter through their action on protein kinase C (25). Tetracycline suppressed the synthesis of TNF-α and IL-1 in human macrophages (19). Recently, it was also reported that doxycycline (1.5 mg/kg) was able to inhibit TNF-α, IL-1, and nitrate secretion in the blood, with a decrease in inducible nitric oxide synthase activity in the spleen and peritoneal cells in a mouse model (13).

The classic primary antibody responses induced by tetanus toxoid, pneumococcal polysaccharide vaccine, and hepatitis B virus vaccine were suppressed by clarithromycin and doxycycline, as evidenced by the IgM levels in the clarithromycin and doxycycline groups being statistically lower than those in the ampicillin and/or NS groups. We speculate that this is partly due to a suppression of the T cell-B cell interaction in the production of antibodies. This is in line with the evidence showing that both clarithromycin and doxycycline can inhibit IL production by T lymphocytes in vitro (15, 19, 22). This is partly analogous to the suppressive effect on vaccination of glucocorticosteroids, which are well-known to down-regulate the production of IL-1, TNF-α, granulocyte-macrophage colony-stimulating factor, IL-3, IL-4, IL-5, IL-8, and inducible nitric oxide synthase (1). However, this cannot fully explain the phenomenon, since antibody production after pneumococcal polysaccharide vaccine administration was also suppressed by both clarithromycin and doxycycline and since the humoral response to pneumococcal polysaccharide vaccine is well-known to be T-cell independent. Other possible targets of action of clarithromycin and doxycycline include antigen presentation, costimulatory signals, and postreceptor events of B-cell activation. Further experiments need to be performed before the exact mechanism can be elucidated.

The suppression by clarithromycin of the antibody response induced by the hepatitis B virus vaccine is persistent, as shown by a persistent suppression of the IgM level at day 28. Moreover, clarithromycin also suppressed the level of IgG1 against HBsAg at day 28, although this did not reach statistical significance. These phenomena were not observed in mice immunized with tetanus toxoid or the pneumococcal polysaccharide vaccine. It would be of both interest and clinical significance to know whether clarithromycin would have the same effect on immunization in humans, especially for HBsAg vaccination. If this is the case, the administration of clarithromycin, like that of glucocorticosteroid (12), cyclosporin A (5), and cytotoxic drugs such as cyclophosphamide (23), would be relatively contraindicated when people receive vaccinations.

There is no conclusive evidence showing any inclination of the immune response toward type Th1 or Th2 in the clarithro-

TABLE 4. Total antibody and antibody subtype levels at days 7 and 21 after intraperitoneal Ty21a-pBR322 administration to mice treated with clarithromycin, doxycycline, ampicillin, or NS

Day postvaccination	Antibody subtype	Serum dilution	Antibody level (A_{492})					
			Mean for group treated with:				SEM (pooled)	Critical value
			Clarithromycin ($n = 10$)	Doxycycline ($n = 10$)	Ampicillin ($n = 10$)	NS ($n = 10$)		
7	Total	1:25	0.096	0.100	0.132[a]	0.069[a]	0.110	0.043
	IgM	1:25	0.226[b]	0.232[c]	0.234[d]	0.122[b,c,d]	0.024	0.093
	IgG1	1:25	0.012	0.013	0.011	0.013	0.001	0.004
	IgG2a	1:25	0.010	0.010	0.020	0.010	0.004	0.016
21	Total	1:25	0.681	0.523[e]	0.973[e,f]	0.302[f]	0.101	0.386
	IgM	1:25	0.482[g]	0.524[h]	0.472[i]	0.158[g,h,i]	0.043	0.162
	IgG1	1:25	0.017	0.015	0.012	0.012	0.002	0.007
	IgG2a	1:25	0.019	0.017	0.019	0.017	0.004	0.016

[a–f] The difference is statistically significant compared to the mean with the same superscript.

TABLE 5. Serum IL-10 levels after subcutaneous tetanus toxoid, subcutaneous pneumococcal polysaccharide vaccine, intraperitoneal HBsAg, or intraperitoneal Ty21a-pBR322 administration to mice treated with clarithromycin, doxycycline, ampicillin, or NS

Vaccine	Day postvaccination	Serum IL-10 level (pg/ml)					
		Mean for group treated with:				SEM (pooled)	Critical value
		Clarithromycin (n = 10)	Doxycycline (n = 10)	Ampicillin (n = 10)	NS (n = 10)		
Tetanus toxoid	7	18	23	21	17	9	33
	21	Undetectable[a]	Undetectable	Undetectable	Undetectable		
	28	55	26	56	88	26	98
Pneumococcal polysaccharide	7	5	4	4	13	3	13
	21	137	32	63	27	44	167
	28	17	12	16	17	9	35
HBsAg	7	31	31	40	49	17	63
	21	15	35	35	40	15	58
	28	2	12	4	2	2	8
Ty21a	7	Undetectable	Undetectable	Undetectable	Undetectable		
	21	Undetectable	Undetectable	Undetectable	Undetectable		

[a] Undetectable, no statistically significant difference between the serum IL-10 levels on that day and day −1.

mycin or doxycycline groups. Although clarithromycin, and to a lesser extent doxycycline, suppressed the level of IgG1 against HBsAg on days 21 and 28 (not statistically significant), no effect on this antibody subclass was found with respect to the other vaccines. Furthermore, for all four vaccines, no difference in the IL-10 or IFN-γ levels can be shown among the mice administered the various antibiotics.

Paradoxically, the antibody responses induced by Ty21a were enhanced by clarithromycin and doxycycline, despite the immunosuppressive effect of these two antibiotics. Furthermore, the antibody response was also enhanced by ampicillin, which is not known to have any immunomodulating effects and has been shown in this study not to affect the antibody response induced by tetanus toxoid, pneumococcal polysaccharide vaccine, or hepatitis B virus vaccine. There is evidence showing that the antibody response of mice against *Escherichia coli* and the protection against wild-type *E. coli* challenge can be augmented by culturing live attenuated *E. coli* in the presence of aztreonam before immunization. The author speculated that this might be due to the partial damage of the bacteria by a sublethal dose of aztreonam, rendering the organisms more immunogenic (9). In our experiments, daily administration of antibiotics to the mice could have also sublethally damaged the Ty21a, making it more immunogenic and therefore inducing an enhanced antibody response. Moreover, the total antibody level of the ampicillin group on day 21 was the highest among all the groups, significantly higher than that of the doxycycline group. This can be explained by the absence of an immunosuppressive effect of ampicillin, such that the antibiotic's immunogenic effect acts on its own. Since the clinical efficacy of the Ty21a vaccine is only 70% in humans (20, 24), the present observation could be important for enhancing the efficacy of the vaccine.

In conclusion, clarithromycin and doxycycline suppress the antibody response induced by tetanus toxoid, pneumococcal polysaccharide vaccine, and HBsAg through their immunomodulating effects, while ampicillin, clarithromycin, and doxycycline enhance the antibody response induced by Ty21a. This may be due to the antibiotic's immunogenic effect, which may overwhelm the immunomodulating effect of clarithromycin and doxycycline. Although the exact mechanism of suppression and enhancement of the antibody response remains to be elucidated, the present observations should prompt further investigation of the practical significance of such phenomena in terms of clinical implications and applications.

TABLE 6. Serum IFN-γ levels after subcutaneous tetanus toxoid, subcutaneous pneumococcal polysaccharide vaccine, intraperitoneal HBsAg, or intraperitoneal Ty21a-pBR322 administration to mice treated with clarithromycin, doxycycline, ampicillin, or NS

Vaccine	Day postvaccination	IFN-γ level (pg/ml)					
		Mean for group treated with:				SEM (pooled)	Critical value
		Clarithromycin (n = 10)	Doxycycline (n = 10)	Ampicillin (n = 10)	NS (n = 10)		
Tetanus toxoid	7	135	180	134	240	76	289
	21	705	1,006	943	620	401	1,526
	28	956	350	390	480	242	923
Pneumococcal polysaccharide	7	769	654	1,047	1,600	534	2,035
	21	Undetectable[a]	Undetectable	Undetectable	Undetectable		
	28	Undetectable	Undetectable	Undetectable	Undetectable		
HBsAg	7	760	1,032	857	570	352	1,340
	21	Undetectable	Undetectable	Undetectable	Undetectable		
	28	678	943	845	460	325	1,236
Ty21a	7	1,523	704	1,045	645	435	1,655
	21	310	212	1,201	625	261	995

[a] Undetectable, no statistically significant difference between the serum IFN-γ levels on that day and day −1.

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